Growth and Ectomycorrhizal Development of Loblolly Pine Seedlings in Fumigated and Nonfumigated Nursery Soil Infested with Different Fungal Symbionts

Donald H. Marx
William G. Morris
John G. Mexal

ABSTRACT. Vegetative mycelial inoculum (mycelium) of *Pisolithus tinctorius, Thelephora terrestris*, and *Cenococcum graniforme*, and three quantities of basidiospores (spores) of *P. tinctorius* were used to infest fumigated and nonfumigated soil in an Oklahoma nursery to ascertain their significance in correcting an earlier deficiency of ectomycorrhizae on seedlings of *Pinus taeda*. All treatments in fumigated soil induced earlier ectomycorrhizal development on *P. taeda* seedlings than found on controls, and they produced a significant increase in the number (75 to 155 percent) and total fresh weights (24 to 125 percent) of plantable seedlings. Seedlings in nonfumigated control plots also formed ectomycorrhizae earlier than those in fumigated control plots, increasing the number of plantable seedlings by 129 percent and their weight by 59 percent. Mycelium of *P. tinctorius* and *T. terrestris* were the only treatments to increase the number of plantable seedlings in nonfumigated plots; no treatment in nonfumigated soil increased seedling weights. Inocula of all fungi were more effective in forming ectomycorrhizae in fumigated than in nonfumigated soil. *C. graniforme* was the least effective in forming ectomycorrhizae and stimulating seedling growth. Mycelium of *P. tinctorius* formed nearly twice as many ectomycorrhizae as did spores; quantity of spores did not influence the amount of *P. tinctorius* ectomycorrhizae. These findings are discussed in regard to their implications for growing pine seedlings in nurseries. FOREST SCI. 24:193–203.

ADDITIONAL KEY WORDS. Reforestation, pine seedling quality, *Pisolithus tinctorius, Thelephora terrestris, Cenococcum graniforme, Pinus taeda*.

THE NEED OF FOREST TREES, especially pines, for ectomycorrhizal associations was first observed when experimental plantations of exotic pines in different parts of the world consistently failed, until suitable ectomycorrhizal fungi had been introduced (Mikola 1973). The absence of ectomycorrhizal fungi in former treeless areas of the United States, such as the Great Plains, has also been widely reported (Hatch 1936, McComb 1938, White 1941, Goss 1960). In most incidences, ectomycorrhizal fungi were introduced into nursery soils in the form of duff or the upper layer of mineral soil from a healthy, mature forest. This method was reliable but costly

and potentially dangerous, particularly in regard to the possibility of introducing harmful microorganisms and noxious weeds.

A recent example of ectomycorrhizal deficiency on pine was detected in 1973 in Oklahoma. Weyerhaeuser Company established a new nursery near Fort Towson in southeastern Oklahoma on former pasture land in an area surrounded by only a few widely scattered trees in shelterbelts and woodlots. In May 1974, the first crop of loblolly pine (*Pinus taeda* L.) seedlings was seeded on 10 acres of nonfumigated soil previously used to grow watermelons. This sowing date was at least a month later than the average sowing date for loblolly pine in this section of the United States. During the growing season, various pesticides were used to control diseases, insects and weeds. Inorganic fertilizers were added to maintain a high level of soil fertility. By late July, very few of the nearly seven million seedlings had produced secondary needles and most were stunted and chlorotic. Root evaluation made at this time revealed that less than 10 percent of the seedlings had ectomycorrhizae. These latter seedlings, however, exhibited secondary needle development. By mid-August, thousands of seedlings were dying each week. Additional fungicides and fertilizers were applied through September without apparent beneficial effects. Seedlings became dormant in late November and were lifted in January 1975. Examinations of sample seedlings showed that only 4 percent had stem diameters greater than 3 mm, the minimum requirement for outplanting; none met the 15-cm height requirement. Few seedlings had ectomycorrhizae.

It was concluded that the poor growth of these seedlings resulted from an insufficient quantity of ectomycorrhizae. Apparently, in the immediate vicinity there were not enough trees, i.e., species of *Pinus*, *Quercus*, *Carya*, *Betula*, *Fagus*, or *Salix*, harboring ectomycorrhizal fungi for such fungi to be spread to the nursery soil by basidiospores, which serve as inoculum for development of ectomycorrhizae. In an effort to increase the availability of inoculum of ectomycorrhizal fungi near the nursery, loblolly pine seedlings with abundant ectomycorrhizae were planted at various locations around the nursery in the fall of 1974. It was hoped that this would increase the likelihood of natural colonization by ectomycorrhizal fungi in the nursery's fumigated soil and thus benefit subsequent seedling crops as has occurred in the past (Mikola 1973).

Recently, techniques were developed for artificially introducing the ectomycorrhizal fungi *Pisolithus tinctorius* (Pers.) Coker and Couch and *Thelephora terrestris* Ehrh. ex Fr. into fumigated nursery soils of the southern United States (Marx and Bryan 1975, Marx and others 1976, Marx and Artman 1978). In such soils, the development of ectomycorrhizae by the introduced fungi significantly improved ectomycorrhizal development and growth of several species of pines. Vegetative mycelial inoculum (mycelium) was a very efficient type of inoculum for obtaining maximum ectomycorrhizal development. Although basidiospores of *P. tinctorius* were also used in several tests, they proved to be less effective. All of these experiments were done in nurseries where there was highly efficient colonization of fumigated soil by basidiospores of naturally occurring ectomycorrhizal fungi. Consequently, within a few weeks after seed germination, ectomycorrhizal development usually had begun, and by the end of the growing season all seedlings were heavily ectomycorrhizal. Results from one of these experiments (Marx and others 1976) strongly indicated that effective soil fumigation must precede artificial soil infestation with symbiotic fungi. It is not known, however, whether fumigation is necessary to reduce populations of all microorganisms in the soil or simply to reduce populations of indigenous, competitive ectomycorrhizal fungi.

The apparent deficiency of ectomycorrhizal fungi at the Oklahoma nursery furnished several unique research opportunities. These include the chance to test

further the practical value of the introduction of pure cultures of ectomycorrhizal fungi into nursery soil, to study the use of pure cultures to correct an apparent deficiency of ectomycorrhizal fungi in a nursery, and to ascertain the significance of fumigation of a nursery soil which contains an apparent low amount of inoculum of ectomycorrhizal fungi that can compete with introduced inoculum.

The purpose of this experiment was to introduce *P. tinctorius*, *T. terrestris*, and *Cenococcum graniforme* (Sow.) Ferd. and Winge as mycelium and *P. tinctorius* as basidiospore inoculum (spores) into fumigated and nonfumigated soil in order to determine their effects on growth and ectomycorrhizal development of loblolly pine seedlings. *C. graniforme* was included in the test because it is drought tolerant (Worley and Hacskaylo 1959, Meyer 1964, Mexal and Reid 1974) and may have the potential to improve the field performance of loblolly pine seedlings planted on droughty sites.

MATERIALS AND METHODS

Inoculum Preparation.—For production of mycelium, *P. tinctorius* (isolate 138), *T. terrestris* (isolate 201), and *C. graniforme* (isolate 146) were grown at room temperature in 1.5 liter volumes of a vermiculite and peat moss mixture saturated with a nutrient medium (Marx and Bryan 1975). After 3 months, *P. tinctorius* and *T. terrestris* had completely colonized all the mixture with mycelia and the total volume of mixture containing these fungi was used as mycelium. However, the slower growing *C. graniforme* did not colonize all the mixture in 3 months; therefore, only that part of the mixture with obvious mycelium of *C. graniforme* was used as mycelium. The mycelium of the different fungi was leached (Marx and Bryan 1975), standardized in volume, placed in plastic bags, and stored in insulated containers maintained between 5° and 12°C for 5 days before used.

Basidiospores of *P. tinctorius* were extracted (Marx 1976a) from several basidiocarps found under mature loblolly pines in Clarke County, Georgia, in the late summer of 1973 and 1974. Immediately after extraction, basidiospores were thoroughly mixed and stored in amber glass bottles at 5°C. Spores were transported in plastic bags and maintained at 5° to 12°C until used.

Nursery Soil Management.—The experiment was installed in a new nursery section which previously had been a pasture supporting a few scattered hardwood trees. In December 1974, 2.5 cm of 2-year-old pine-hardwood sawdust was disked into the soil. In early January 1975, 1,500 kg/ha of NH_4NO_3 and 525 kg/ha of 12-24-12 fertilizer were broadcast and disked into the soil, and in late January an additional 1,000 kg/ha of NH_4NO_3 were similarly applied. In early March, 612 kg/ha of 0-18-36 fertilizer and 13.4 kg/ha of Captan® (50 percent wettable powder) were also incorporated.

Plot Installation.—Fourteen test plots, each 3.6 m long × 1.2 m wide, were placed 7 m apart in each of five nursery beds. Each nursery bed was 7.5 m from the irrigation lines. Soil samples were collected from each of the 70 test plots and assayed within 4 hours for *Pythium* and *Phytophthora* species and within 7 days for plant-parasitic nematodes (Marx and others 1976). Chemical and mechanical analyses were also made of each soil sample.[1]

Seven test plots per bed were selected at random, and the entire 3.6 m × 1.2 m area was covered with clear plastic and then fumigated with methyl bromide (0.24 kg/■ MC-2, Dow Chemical Company). Soil temperature (19° to 28°C) and

plots or 540 kg/ha;

[1] Chemical and mechanical analyses of soil samples were performed by the Soil Testing and Plant Analysis Laboratory, Cooperative Extension Service, University of Georgia, Athens 30602.

moisture (40 to 50 percent of field capacity) were ideal for effective soil fumigation. The plastic was removed after 15 days, and the soil was allowed to vent for an additional 4 days before it was infested with the test symbionts.

Soil Infestation and Planting.—Twenty-four hundred ml of mycelium of *P. tinctorius*, *T. terrestris*, and *C. graniforme* were broadcast evenly (1,080 ml/m²) over the middle 1.8 m × 1.2 m area of randomly selected plots and incorporated into the upper 12 cm of soil with handtools. Spores of *P. tinctorius* were added to 2,400 ml of moist vermiculite in plastic bags, mixed thoroughly by hand, broadcast onto the soil, and mixed into the soil with handtools. The spore quantities were 108, 324, or 648 mg of basidiospores per m² of soil surface. There are approximately 1.1 million spores per mg. The 324 mg/m² rate of *P. tinctorius* spores is comparable to that which synthesized the greatest amount of ectomycorrhizae on loblolly pine in earlier tests (Marx 1976a). Control plots received 2,400 ml of moist, nonamended vermiculite incorporated as above. The 0.9 m long × 1.2 m wide fumigated strip on both ends of each of the fumigated plots was designed to minimize contamination of the artificially infested soil by the adjacent nonfumigated soil in the nursery bed. The experimental plots, therefore, were each 1.8 m long × 1.2 m wide and were separated from each other by 8.8 m of nonfumigated nursery bed.

The experimental design was a randomized block. Each of the five nursery beds was a replicate block containing seven fumigated and seven nonfumigated plots. Each of the seven ectomycorrhizal treatments, i.e., mycelium of *P. tinctorius*, *T. terrestris*, and *C. graniforme*, spores of *P. tinctorius* at 108, 324, and 648 mg of basidiospores/m² of soil surface, and the control, were randomly assigned to seven fumigated and seven nonfumigated plots per block.

Each nursery bed was row-planted in its entirety with Arasan®-treated seed of loblolly pine (1974 crop from a Mississippi-Alabama source) and mulched with 1,340 kg/ha of hydromulch (Silva-fiber, Weyerhaeuser Company, with 2.4 percent by volume of Petroset SC, Phillip's 66, as a sticker). Installation of the experiment was completed on April 23, 1975.

During the growing season, all plots were fertilized uniformly with nitrogen (N) as NH_4NO_3 and potassium (K) as KCl according to the following schedule: May, 16.4 kg of N/ha; June, 33.2 kg of N/ha; July, 39.1 kg of N/ha; September, 24.4 kg of N/ha; and in October, to promote cold hardening of the seedlings, 33.6 kg of K/ha. All fertilizers were broadcast, and the irrigation system was used to wash away any fertilizer adhering to the seedlings. Approximately 75 cm of irrigation water was applied to seedlings during the growing season to augment rainfall. Periodically throughout the growing season, several seedlings were removed from each plot and their roots examined for ectomycorrhizae. Incidence of *P. tinctorius* and *T. terrestris* basidiocarps were recorded; since seedlings can be damaged by the removal of *T. terrestris* basidiocarps from their stems, only basidiocarps of *P. tinctorius* were removed from the plots.

Two weeks before the seedlings were lifted in early December, all test plots were laterally root pruned. Just prior to lifting, all plots were undercut with a root-pruning bar at a depth of 10 to 12 cm. All test seedlings were removed from each plot by hand, counted and graded, and the number of *T. terrestris* basidiocarps was recorded. Seedlings less than 12.5 cm in height or with stems less than 3 mm in diameter were counted but discarded as cull (nonplantable) seedlings. Twenty-five plantable seedlings from each plot were chosen at random for measurements of stem height, stem diameter at the root collar, and fresh weights of shoots and roots. The degree of ectomycorrhizal development was estimated visually (Marx and others 1976). Five-hundred typical ectomycorrhizae formed by each of *P. tinctorius*, *T. terrestris*, and *C. graniforme* were removed from seedlings, surface sterilized for 3

minutes in 100 ppm of HgCl₂ solution, rinsed several times in sterile water, plated individually into tubes of modified Melin-Norkrans agar medium, and incubated at 25°C (Marx and others 1976). Fungal cultures obtained from the ectomycorrhizae were compared with the original isolates of *P. tinctorius*, *T. terrestris*, or *C. graniforme* for confirmation of the identity of the new cultures.

Analyses of variance were made on all data, and differences among treatment means were evaluated with Duncan's Multiple Range Test ($P = 0.05$). Two-way comparison of treatment means was done with analysis of variance for comparing parameters from fumigated to nonfumigated plots.

RESULTS

Prior to infestation, soil in the test plots contained an average (in kg/ha) of 58 for available P, 150 for exchangeable K, 453 for exchangeable Ca, and 54 for NO₃-N. This sandy loam soil had a pH of 5.9 and contained 0.6 percent organic matter. Apparently, the nearly 900 kg/ha of N, and 240 kg/ha of P and K applied to the sawdust-amended soil prior to study installation stimulated rapid decomposition of the sawdust to a very low level, i.e., 0.6 percent. Additionally, most of the added elements, especially N, obviously leached from this sandy loam soil. Fewer than one propagule of *Pythium* spp. per gram of soil was isolated from any soil sample, and no plant-parasitic nematodes were found in any sample taken at the time of study installation.

By mid-June, a few ectomycorrhizae typical in morphology of those formed by *P. tinctorius* and *T. terrestris* (Marx and Bryan 1975) were first observed in the fumigated and nonfumigated plots that had been infested with mycelium of *P. tinctorius* and *T. terrestris*. Seedlings in control plots, as well as those in plots treated with *C. graniforme* and spores of *P. tinctorius*, were stunted and chlorotic and had only occasional ectomycorrhizae.

By mid-August, basidiocarps of *T. terrestris* and *P. tinctorius* were observed in their respective mycelium treatments. *T. terrestris* basidiocarps were on stems of about 5 percent of the seedlings in mycelium plots of *T. terrestris* in both fumigated and nonfumigated soil, whereas less than 0.1 percent of the seedlings in other plots had *T. terrestris* basidiocarps. Seedlings in fumigated and nonfumigated soil with mycelium of *P. tinctorius* and *T. terrestris* were dark green in color and visually at least twice as large as the chlorotic and stunted seedlings in the fumigated and non-fumigated plots of the controls or with *C. graniforme* or spores of *P. tinctorius*. Only a few of the seedlings in the latter treatments had ectomycorrhizae. Approximately 35 to 40 percent of the short roots were ectomycorrhizal by *P. tinctorius* and *T. terrestris* in their respective mycelium plots in both fumigated and nonfumigated soil. The typical black *C. graniforme* ectomycorrhizae were occasionally observed in the mycelium of *C. graniforme* treatments in fumigated and nonfumigated soil.

In early October, seedlings in the control plots and those inoculated with spores or mycelium of *C. graniforme* in nonfumigated soil began to grow at a rate comparable to seedlings in the nonfumigated plots of the other treatments. This growth change was accompanied by a change in foliage color from chlorotic to green. Seedlings of these treatments in fumigated soil were still growing slowly but were beginning to lose some of their chlorotic appearance. Associated with this increase in growth and disappearance of chlorosis was the appearance of more *T. terrestris* basidiocarps in the control plots and those inoculated with spores or mycelium of *C. graniforme* in both fumigated and nonfumigated soil.

After the seedlings were lifted, *P. tinctorius* was reisolated from 48 percent, *T. terrestris* from 18 percent, and *C. graniforme* from 24 percent of their respective ectomycorrhizae selected as morphologically representative of those formed by each
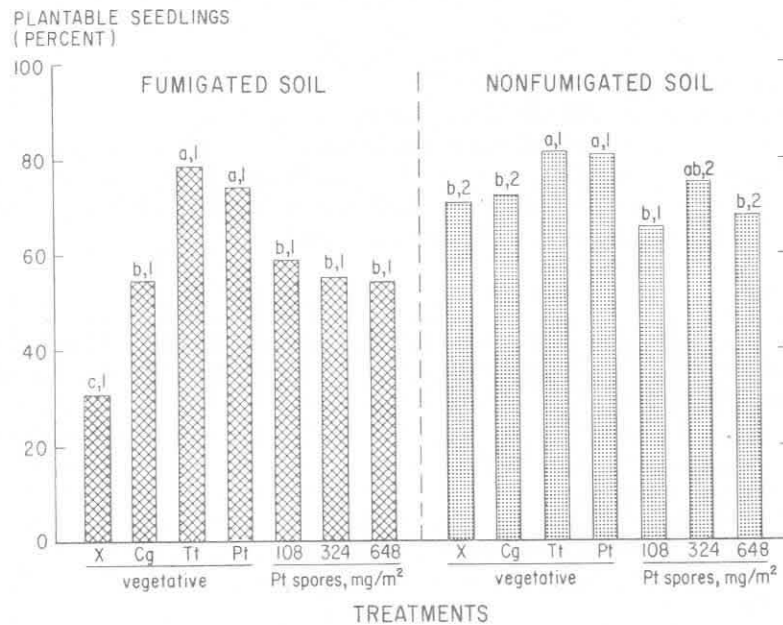
FIGURE 1. Percent of loblolly pine seedlings of plantable size from fumigated and nonfumigated soil artificially infested with different ectomycorrhizal fungi. Within fumigation treatments, columns with a common letter are not significantly different at $P = 0.05$; common numbers indicate that the same fungus treatment is not significantly different in the two fumigation treatments. (X = control, Cg = *Cenococcum graniforme*, Tt = *Thelephora terrestris*, Pt = *Pisolithus tinctorius*.)

fungus. Other ectomycorrhizal fungi were not isolated from these specific ectomycorrhizae.

Over 54,000 seedlings were lifted and evaluated in December. Density of seedlings ($\bar{x} = 358$ seedlings/m^2) was not significantly affected by soil fumigation or by ectomycorrhizal fungus treatment.

In the fumigated soil, basidiocarps of *T. terrestris* were found on 44 percent of the seedlings from the treatment with mycelium of *T. terrestris* and on only 0.8 to 3.5 percent of the seedlings from the other treatments. In the nonfumigated soil, 43 percent of the seedlings from the treatments with mycelium of *T. terrestris* and only 1.3 to 5.0 percent of seedlings from the other treatments had basidiocarps of *T. terrestris*. Basidiocarps of *P. tinctorius* were observed in all plots treated with mycelium and spores in both fumigated and nonfumigated soil, but not in other plots. Basidiocarps of other fungi were not detected in any plot.

In fumigated soil, the number of plantable seedlings was increased by 77 percent over the controls with mycelium of *C. graniforme*, by 155 percent with mycelium of *T. terrestris*, by 140 percent with mycelium of *P. tinctorius*, and by 81, 79, and 75 percent by the three spore treatments of *P. tinctorius* (Fig. 1). Only mycelium of *T. terrestris* and *P. tinctorius* increased the number of plantable seedlings in nonfumigated soil over that in the nonfumigated control plots. Nonfumigated control plots had 129 percent more plantable seedlings than fumigated control plots.

Height, stem diameter, and fresh weights of tops and roots of plantable seedlings were generally increased by all the ectomycorrhizal fungus treatments in fumigated soil (Fig. 2). Mycelium of *T. terrestris* and *P. tinctorius* increased total fresh
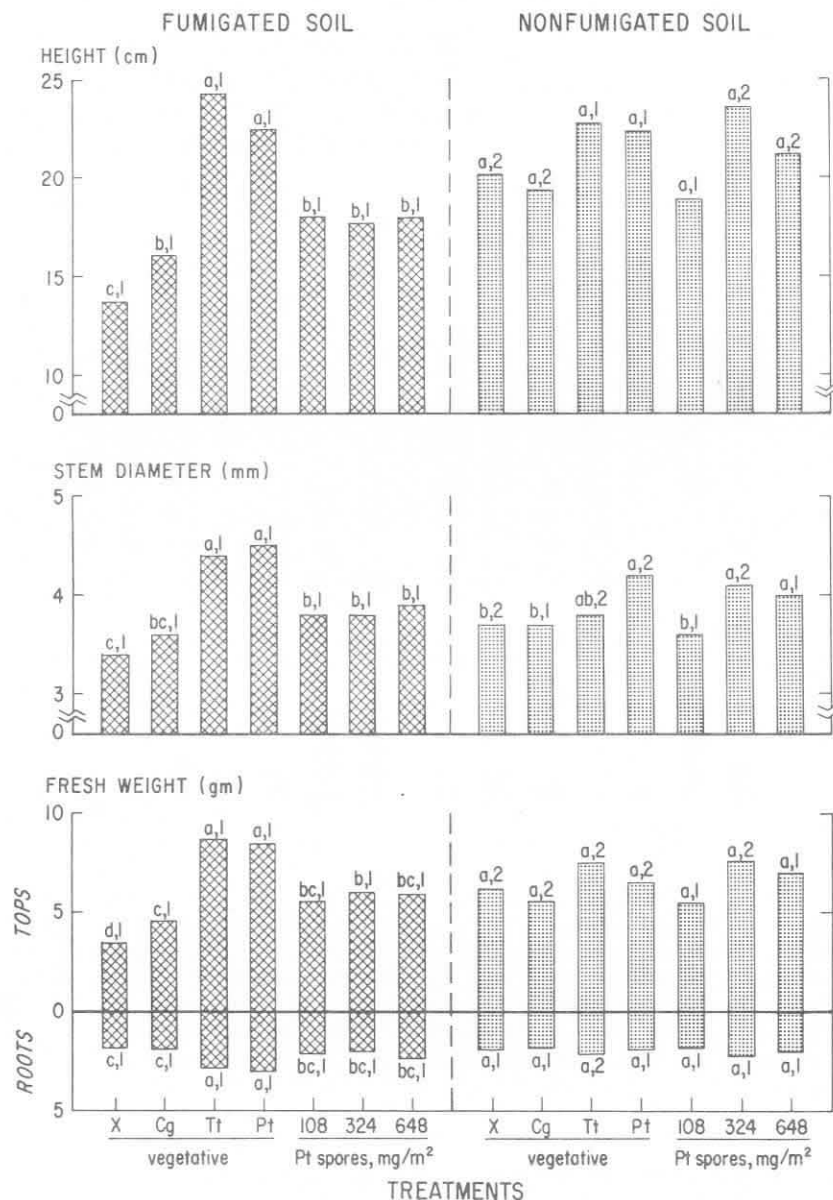
FIGURE 2. Growth measurements of loblolly pine seedlings grown in fumigated and nonfumigated soil artificially infested with different ectomycorrhizal fungi. Within fumigation treatments, columns with a common letter are not significantly different at $P = 0.05$; common numbers indicate that the same fungus treatment is not significantly different in the two fumigation treatments. (X = control, Cg = *Cenococcum graniforme*, Tt = *Thelephora terrestris*, Pt = *Pisolithus tinctorius*.)

weights of seedlings by approximately 125 percent. Spores of *P. tinctorius* treatments increased total fresh weight by 51 to 61 percent, and mycelium of *C. graniforme* increased seedling weights by 24 percent. Fresh weight was 59 percent greater for seedlings from nonfumigated control plots than for these seedlings from
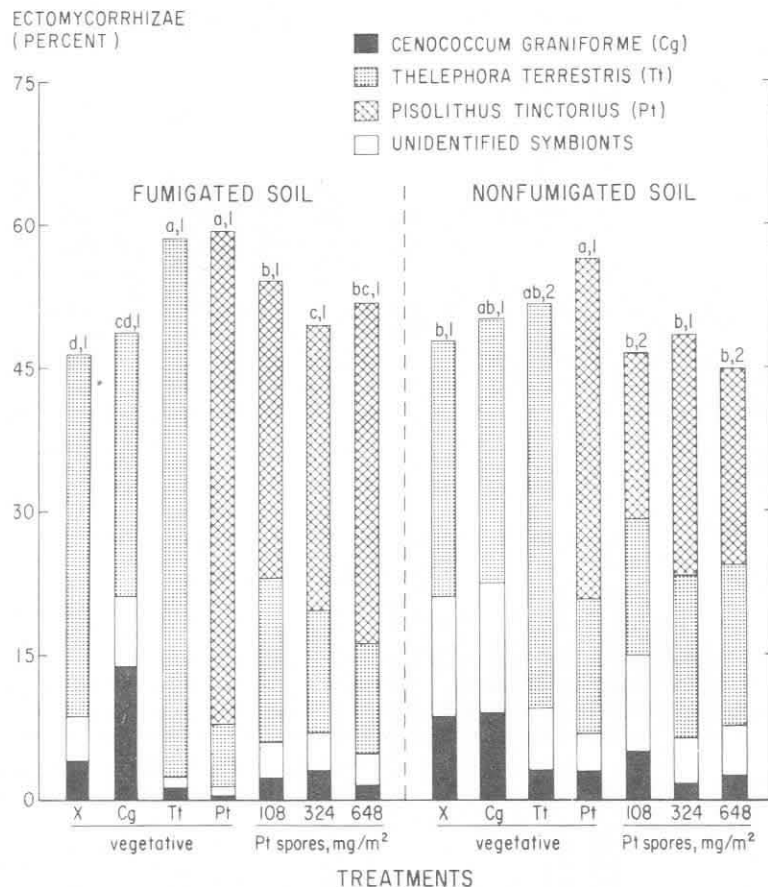
FIGURE 3. Total percent of feeder roots with ectomycorrhizae and percent of specific ecto-mycorrhizae formed on loblolly pine seedlings in fumigated and nonfumigated soil artificially infested with different ectomycorrhizal fungi. Within fumigation treatments, columns with a common letter are not significantly different at $P = 0.05$; common numbers indicate that the same fungus treatment is not significantly different in the two fumigation treatments. (X = control, Cg = *Cenococcum graniforme*, Tt = *Thelephora terrestris*, Pt = *Pisolithus tinctorius*.)

fumigated control plots. None of the fungus treatments improved height or fresh weight of seedlings in nonfumigated plots.

Ectomycorrhizal development by specific fungi was strongly affected by fumigation and soil infestation (Fig. 3). In fumigated plots, naturally occurring *C. graniforme* accounted for only about 10 percent of all the ectomycorrhizae formed on seedlings in plots other than the mycelium of *C. graniforme*. In the latter treatment, *C. graniforme* accounted for nearly 35 percent of the ectomycorrhizae. However, the percentage of *C. graniforme* ectomycorrhizae in fumigated plots treated with mycelium of *C. graniforme* was not different from that found on control seedlings in nonfumigated plots.

T. terrestris dominated the roots of seedlings from the treatment with mycelium of *T. terrestris* in both fumigated and nonfumigated soil. There was, however, much naturally occurring *T. terrestris* on seedlings in the other treatments. Seedlings from

the treatment with mycelia of *T. terrestris* in fumigated soil had a higher percentage of ectomycorrhizae than did seedlings from any other treatment except the mycelium of *P. tinctorius*.

P. tinctorius from mycelium in fumigated soil accounted for nearly 90 percent of all the ectomycorrhizae formed on seedlings and approximately 70 percent of all the ectomycorrhizae in the treatments with the three quantities of spores in fumigated soil. However, *P. tinctorius* dominance was somewhat less in the nonfumigated plots, although it still formed the majority of the ectomycorrhizae.

Ectomycorrhizae formed by unidentified fungal symbionts accounted for approximately 8 percent of all the ectomycorrhizae formed on seedlings in fumigated plots and approximately 17 percent of those formed in the nonfumigated soil.

DISCUSSION

Numerous basidiocarps of *T. terrestris* were observed throughout the growing season under the loblolly pine seedlings that had been planted to increase the inoculum potential of the ectomycorrhizal fungi near the nursery. Wind-disseminated basidiospores from these basidiocarps and those produced in test plots of *T. terrestris* undoubtedly accounted for the occurrence of *T. terrestris* on roots of the loblolly pine seedlings in this experiment. Soil fumigation obviously decreased the quantity of ectomycorrhizal fungus inoculum since control seedlings in fumigated soil did not form appreciable ectomycorrhizae until late in the growing season (October). The earlier appearance and greater incidence of naturally occurring *Cenococcum* and other unidentified fungal symbionts on seedlings from nonfumigated soil also support this conclusion. The residual inoculum in the nonfumigated soil, however, apparently was not evenly distributed since much greater variation in seedling growth within and between plots of the same treatment occurred in nonfumigated soil than in fumigated soil.

Vegetative mycelial inoculum proved again (Marx and others 1976) to be an excellent form of inoculum for early and continued development of ectomycorrhizae on pine seedlings in nurseries. The early and prolific development of ectomycorrhizae on seedlings from these treatments in both fumigated and nonfumigated soil corresponded well with stimulation of seedling growth and quality. This correspondence was especially noticeable in the fumigated soil. As observed earlier with *P. tinctorius* (Marx and Bryan 1975, Marx and others 1976), basidiospores are not as effective as mycelium for early development of ectomycorrhizae. Stimulation of seedling growth in the basidiospore treatments was correlated with the appearance of *Pisolithus* ectomycorrhizae on roots. It is obvious that soil fumigation reduces the inoculum potential of indigenous symbiotic fungi and perhaps other competing microorganisms and thereby increases the ability of basidiospores to synthesize ectomycorrhizae; all quantities of basidiospores formed nearly 50 percent more ectomycorrhizae in fumigated than in nonfumigated soil. There was not a well-defined difference in percentages of *Pisolithus* ectomycorrhizae formed by different quantities of basidiospores. Because of within-treatment variation in the percentage of ectomycorrhizae formed, 108 mg of basidiospores/m^2 were as effective as 648 mg/m^2.

Our results indicated that *C. graniforme* is not suitable for artificial introduction into either fumigated or nonfumigated soil in this nursery. Apparently, its inherently slow growth rate, as well as its greater tolerance for droughty soil conditions (Worley and Hacskaylo 1959, Meyer 1964, Mexal and Reid 1974), does not allow it to compete effectively against other naturally occurring symbiotic fungi which may be better adapted to the irrigated soils in this nursery. Perhaps with maintenance of

less soil moisture, this fungal symbiont could be effectively maintained on seedling roots.

The value of ectomycorrhizae to growth of pines has been demonstrated many times (Marks and Kozlowski 1973). It is worthy here, however, to point out that even with high levels of N, P, K, and Ca in soil at sowing, the absence of phycomyceteous root pathogens and nematodes, and the application of nearly 120 kg/ha of N during the major part of the growing season, seedlings in noninfested fumigated soil did not begin to grow appreciably until ectomycorrhizae naturally formed on their roots. The effect of ectomycorrhizae was for such a short duration, however, that those seedlings were really valueless as outplanting stock at lifting time. These results show that early ectomycorrhizal development on loblolly pine seedlings is an essential prerequisite to improving the number of plantable seedlings and their size. In certain geographic regions, such as southeastern Oklahoma, where naturally occurring inoculum of ectomycorrhizal fungi may be deficient or erratic in occurrence, soil infestation with symbiotic fungi following soil fumigation is essential to the consistent production of quality pine seedlings needed to meet forestation needs. This report, however, proves that pure cultures of specific ectomycorrhizal fungi can be used to correct this deficiency. Certain of these fungi, such as *P. tinctorius*, can not only increase seedling production in these deficient nurseries but also increase their field performance on adverse (Marx 1976b) and routine (Marx and others 1977) reforestation sites.

LITERATURE CITED

Goss, R. W. 1960. Mycorrhizae of ponderosa pine in Nebraska grassland soils. Univ Neb Res Bull 192, 47 p.

Hatch, A. B. 1936. The role of mycorrhizae in afforestation. J For 34:22–29.

McComb, A. L. 1938. The relation between mycorrhizae and the development and nutrient absorption of pine seedlings in a prairie nursery. J For 36:1148–1155.

Marks, G. C., and T. T. Kozlowski. 1973. Ectomycorrhizae—Their ecology and physiology. Academic Press, New York. 444 p.

Marx, D. H. 1976a. Synthesis of ectomycorrhizae on loblolly pine seedlings with basidiospores of *Pisolithus tinctorius*. Forest Sci 22:13–20.

Marx, D. H. 1976b. Use of specific mycorrhizal fungi on tree roots for forestation of disturbed lands. *In* Proc Conf on Forestation of Disturbed Surface Areas, Birmingham, Alabama, April 14–15, 1976, p 47–65. USDA Forest Service, State and Private Forestry, and International Forest Seed Company.

Marx, D. H., and J. D. Artman. 1978. Growth and ectomycorrhizal development of loblolly pine seedlings in nursery soil infested with *Pisolithus tinctorius* and *Thelephora terrestris* in Virginia. USDA Forest Serv Res Note SE-256, 4 p.

Marx, D. H., and W. C. Bryan. 1975. Growth and ectomycorrhizal development of loblolly pine seedlings in fumigated soil infested with the fungal symbiont *Pisolithus tinctorius*. Forest Sci 21:245–254.

Marx, D. H., W. C. Bryan, and C. E. Cordell. 1976. Growth and ectomycorrhizal development of pine seedlings in nursery soils infested with the fungal symbiont *Pisolithus tinctorius*. Forest Sci 22:91–100.

Marx, D. H., W. C. Bryan, and C. E. Cordell. 1977. Survival and growth of pine seedlings with *Pisolithus* ectomycorrhizae after two years on reforestation sites in North Carolina and Florida. Forest Sci 23:363–373.

Mexal, J. G., and C. P. P. Reid. 1974. The growth of selected mycorrhizal fungi in response to induced water stress. Can J Bot 51:1579–1588.

Meyer, F. H. 1964. The role of the fungus *Cenococcum graniforme* (Sow.) Ferd. et Winge in the formation of mor. *In* Soil microbiology, (E. A. Jongerius, ed), p 23–31. Elsevier, Amsterdam.

Mikola, P. 1973. Application of mycorrhizal symbiosis in forestry practices. *In* Ectomycor-

rhizae—Their ecology and physiology, (G. C. Marks and T. T. Kozlowski, eds), p 383–411. Academic Press, New York.

WORLEY, J. F., and E. HACSKAYLO. 1959. The effect of available soil moisture on the mycorrhizal association of Virginia pine. Forest Sci 5:267.

WHITE, D. P. 1941. Prairie soil as a medium for tree growth. Ecology 22:398–402.